First insights into karyotype evolution within the family Mormyridae

by

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Abstract. – The chromosomal diversity within six species of Mormyridae belonging to six different genera (*Gnathonemus petersii*, *Marcusenius moorii*, *Ivindomyrus obdenboschi*, *Brienomyrus* sp., *Stomatorhinus walkeri* and *Petrocephalus microphthalmus)* is investigated for the first time. These species have a conserved diploid chromosome number of 2n = 50 (except *G. petersii* 2n = 48) but different chromosome formulae, essentially involving pericentric inversions. C-banding has been used for the localization of heterochromatin. For a deeper interspecific comparison, several molecular markers were localized in the chromosomes by fluorescence *in situ* hybridization (FISH): a 18S rDNA probe; a microsatellite probe (GAA)$_{10,}$ and a telomeric probe (TTAGGG)$_{7.}$ The distribution of the 18S rDNA patterns, in particular, shows an amazing specific diversity, confirming a rapid radiation accompanied by major chromosome rearrangements.

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Résumé. – Première estimation de l'évolution du caryotype chez les Mormyridae.

La diversité chromosomique a été explorée pour la première fois parmi six espèces de Mormyridae appartenant à six genres différents (*Gnathonemus petersii*, *Marcusenius moorii*, *Ivindomyrus obdenboschi*, *Brienomyrus* sp., *Stomatorhinus walkeri* and *Petrocephalus microphthalmus*). Ces espèces ont un nombre identique de chromosomes (2n = 50), à l'exception de *G. petersii* (2n = 48), mais des formules chromosomiques différentes, mettant essentiellement en jeu des inversions péricentriques. Le marquage en bandes C a été utilisé pour localiser l'hétérochromatine. Pour une comparaison interspécifique plus approfondie, plusieurs marqueurs moléculaires ont été localisés dans les chromosomes par hybridation *in situ* en fluorescence (FISH) : une sonde ADN ribosomique 18S; un microsatellite (GAA)$_{10,}$ et une sonde télomérique (TTAGGG)$_7$. La distribution chromosomique des signaux produits par la sonde ADNr 18S est particulièrement diversifiée parmi ces espèces, ce qui indique une radiation rapide accompagnée par des remaniements chromosomiques majeurs.

Key words

Mormyridae
Congo basin
Cytogenetics
Karyotypes
Chromosomes
Fluorescence *in situ* hybridization

The Mormyridae (Osteoglossomorpha) represent one of the largest freshwater fish families endemic to Africa. Currently 216 species in 21 genera are described from freshwater habitats over most of the continent with the exception of the Sahara, Maghreb and southernmost Cape provinces; they are most diverse in the river systems of Central Africa. Adult mormyrids range from about 4 cm to 1.5 m in length and vary considerably in morphology. *Campylomormyrus* and some *Mormyrops* and *Mormyrus* species have long tubular snouts used for extracting invertebrates from sediment (Hopkins, 1986). In the genera *Marcusenius*, *Gnathonemus*, and *Genyomyrus,* the species possess a variously developed fleshy protuberance on the chin that functions in electrolocation of prey organisms (von der Emde, 2006). In all mormyrids, the head (including the eyes), dorsum and belly are covered by a thin layer of skin beneath which lie electroreceptors of different varieties. The electric organ lies within the caudal peduncle.

Most mormyrids are nocturnal invertebrate-feeders. However, adults of some species of the genus *Mormyrops* are piscivorous.

Molecular studies have provided a well-supported tree for the major mormyrid lineages (Alves-Gomes and Hopkins, 1997; Sullivan *et al*., 2002; Lavoué *et al*., 2003) and morphological works and the molecular studies agree on: (1) the monophyly of Mormyridae; (2) the sister-group relation-

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ship between Mormyridae and Gymnarchidae; and (3) the basal division of the family into two subfamilies, Mormyrinae and Petrocephalinae, with the latter containing only *Petrocephalus*. Finally, the Mormyridae generate and sense weak discharges for electrolocating objects and prey in their environment and for communicating. These electric signals may play a role in the reproductive isolation of sympatric species (Sullivan *et al*., 2002; Carlson *et al*., 2011; Lavoué *et al*., 2012), In certain instances, e.g. some *Pollimyrus* species, the female choice prevents panmixia between sibling species complex (Schmid and Kramer, 2014). Electric organ discharges (EODs) recordings have been used in taxonomy complementary to osteological, morphological and meristic characters (reviewed by Hopkins *et al*., 2007) and the necessity for further systematic studies and taxonomic revision of the Mormyridae emphasized. Mormyrid phylogenetic interrelationships have been inferred from cytochrome b sequences (Lavoué *et al.,* 2000), but there was incongruence between the mitochondrial gene tree and the nuclear gene-based phylogeny caused by incomplete mitochondrial lineage sorting and/or introgression. Mormyrid evolution has been described as a rapid radiation, including the first and currently the unique example of a riverine species flock (Gabon, *Brienomyrus* species complex) as opposed to a lacustrine environment (Sullivan *et al*., 2002). In multiple cases, fish morphs with morphological and genetic similarity appear to have alternative electric signals. For example, in the Ogowe River basin, while 38 EOD waveforms have been described, only five species are identified (Sullivan *et al*., 2002). Currently, genetic and morphological tools are insufficient to discriminate the morphs that show different electric waveforms (Arnegard and Carlson, 2005). At the basin scale, the different populations live in sympatric conditions, but at the habitat scale each of them is isolated from the others. So, this "allopatry" may be considered as the motor for the speciation of the complex, as observed in the cichlids in the Great Lakes of East Africa. We can therefore state that the Gabon's *Brienomyrus* species complex is a true riverine species flock (Sullivan *et al*., 2002; Arnegard and Carlson, 2005).

Finally, in the *Paramormyrops* species flock, the signal evolution is rapid compared to divergence in morphology, size, and trophic ecology. This disparity in the tempo of trait evolution suggests that sexual selection is an important early driver of species radiation in mormyrids (Arnegard *et al*., 2010). The cytogenetic information on Mormyridae (Uyeno, 1973) is scarce. It deals only with the chromosome numbers and formulae of two species purchased from tropical fish stores, *Gnathonemus petersii* (Günther, 1862) and *Marcusenius brachyistius* (currently valid name: *Brienomyrus brachyistius* (Gill, 1862)). This paper is a first estimation of chromosomal diversity within six species belonging to six different genera, namely: *Gnathonemus petersii*, *Marcusenius moorii* (Günther, 1867), *Ivindomyrus obdenboschi* Taverne & Géry, 1975, *Brienomyrus* sp., *Stomatorhinus walkeri* (Günther, 1867) and *Petrocephalus microphthalmus* Pellegrin, 1909. Chromosome numbers and formulae have been established from chromosome preparations obtained by direct *in vivo* methods. For species delineation, interspecific comparison and for possibly understanding karyo-evolutionary pathways, diverse classical and molecular cytogenetic techniques have been applied. The chromosomal distribution of constitutive heterochromatin has been studied by the C-banding method (Sumner, 1972). Moreover, several molecular markers could be localized in the chromosomes by fluorescence *in situ* hybridization (FISH): a 18S rDNA probe; a microsatellite oligonucleotide probe $(GAA)_{10,}$ and a telomeric oligonucleotide probe $(TTAGGG)_{7.}$

MATERIALS AND METHODS

Chromosome preparations and specimens under study

Chromosome preparations were obtained from cephalic,

Table I. - List of specimens analyzed, their origins (see also figure 1) and their vouchers. Undet. = undetermined.

Genus / Species	Number of specimens male / female / undet.	Geographic origin	Voucher
Gnathonemus petersii	2 / 0 / 2	Aquarium CO1 sequences = www.boldsystems.org CYTRS001-14 CYTRS002-14	MNHN-2014-154 MNHN-2014-155
Marcusenius moorii	0 / 0 / 7	Ntem River	MNHN-2003-1192
Ivindomyrus opdenboshi	0 / 1 / 1	Ntem River	MNHN-2003-1214
Brienomyrus sp.7	0 / 0 / 2	Ebeigne, Woleu River	CUMV 80885
Stomatorhinus walkeri	3 / 3 / 0 0 / 0 / 1	Lambaréné Ogooué Basin	MNHN-2003-1213 MNHN-2014-2930
Petrocephalus microphthalmus	1 / 0 / 1	Lambaréné	MNHN-2002-0259

kidney and spleen by direct *in vivo* method, according to the protocol of Bertollo *et al.* (1978) with the following modifications: specimens received a lethal dose of ethyl 3-aminobenzoate methanesulfonate salt (Sigma-Aldrich) prior to dissection. Cephalic, kidney and spleen tissues were rapidly dissociated with forceps, on 70 μm mesh cell strainers placed in small Petri dishes containing 2 ml hypotonic solution (0,075M KCl). The cell suspension was transferred into 15 ml conical tubes containing 10 ml of pre-warmed hypotonic solution. The hypotonic treatment was performed 20 min in a water bath at 23°C with regular gentle agitation.

The list of specimens under study and of their vouchers, and their locality of capture are provided in table I and figure 1. Some of the karyotyped specimens could not be preserved as vouchered specimens, since they were used for other scientific purposes. In such cases we designated vouchers from the same catch/locality/identification as reference specimens. Ethical approval for all procedures was granted by the ethics committee of the Ministry of Environment.

Phylogenetic reconstruction

As a baseline for the results and discussion, a simplified phylogenetic tree including the six genera under study and rooted on the sister species of Mormyridae, *Gymnarchus niloticus* has been built from the mitochondrial cytochrome b partial sequences.

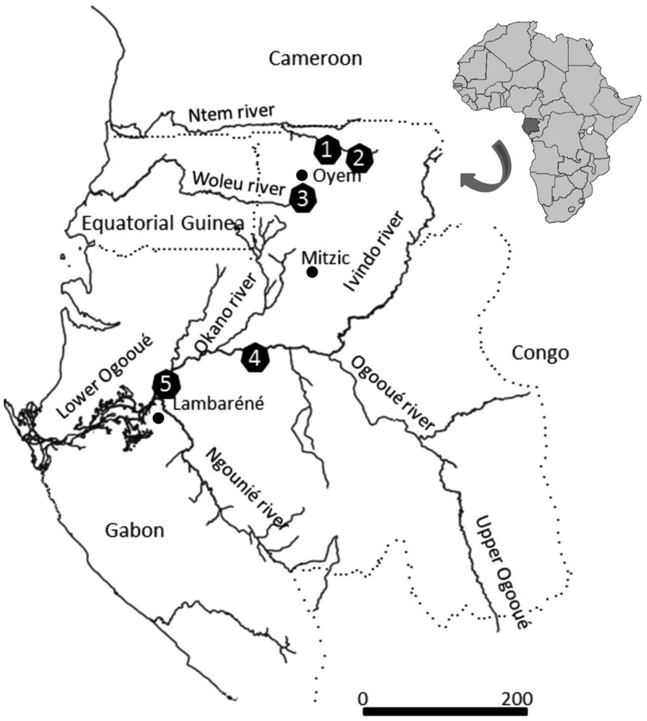

Figure 1. - Map of West-Central Africa showing the five collection sites of the Mormyrid specimens. 1: Ntem River, *M. moori*; 2: Ntem River, *I. opdenboshi*; 3: Woleu River, *B.* sp. 7; 4: Medium Ogooué River, *S. walkeri*; 5: Lower Ogooué River, *P. Microphthalmus*.

Sequences obtained from Genbank were aligned manually using BioEdit version 5.09 (Hall, 1999): AF477479 (*Brienomyrus* sp.), AF201585 (*Gnathonemus petersii*), AF201604 (*Petrocephalus microphthalmus),* DQ166690 (*Ivindomyrus opdenboschi*), AF201595 (*Marcusenius moorii*), AF201586 (*Gymnarchus niloticus*), AP011583 (*Stomatorhinus cf. ater*).

Aligned sequences were analysed using the Maximum Likelihood method using MEGA (Molecular Evolutionary Genetics Analysis) version 5.1 (Kumar *et al.*, 2004). Prior to analysis, an evolutionary model for ML was selected by MEGA 5.1 using the Bayesian information criterion (BIC) (Schwarz, 1978). Support for inferred clades were obtained through non-parametric bootstrap (Felsenstein, 1985) with 2000 replicates

Chromosome numbers and formulae

Mitotic chromosomes were Giemsa stained and karyotypes were established according to the centromere position following the nomenclature of Levan *et al.* (1964). The chromosomes were classified in order of decreasing size and paired only when their morphology made it possible.

C-Banding

The C-banding method for characterization of heterochromatic blocks was performed according to Sumner (1972).

Fluorescence in situ hybridization (FISH)

18S rDNA probe preparation

The 18S rDNA was amplified from a DNA extract of *Paramormyrops sphekodes* (Sauvage, 1879) (voucher specimen deposited in the MNHN, collection number MNHN-2014-2822), using the primers UF15 (5'-CTGCCAGTAGATATGC-3') and UR1765 (5'-ACCTTGTTACCTTTAC-3') (Frischer *et al.*, 2000). Amplification products were separated on 1% agarose gel. The band with the expected molecular weight was excised from the agarose gel, purified with NucleoSpin PCR clean-up columns (Macherey-Nage), cloned in pGEM-T vector according to the manufacturer's recommendations (Promega) and sequenced (GATC Biotech). The probe was labelled with digoxigenin-11-dUTP using nick-translation (Roche) according to the manufacturer's protocol.

Microsatellite and telomeric probes preparation and hybridization

Oligonucleotides containing microsatellite sequences $(CA)_{15}$, $(GA)_{15}$, $(GC)_{15}$, $(CAA)_{10}$, $(CAG)_{10}$, $(GAA)_{10}$ and $(TAA)_{10}$ or telomeric sequence $(TTAGGG)_7$ were purchased (biomers.net, Ulm, Germany), directly labelled with Cy3 or

Atto 488, respectively at their 5' end during their synthesis. Microsatellites oligonucleotides were diluted at 10 ng/μl in the hybridization buffer (65% formamide, 2 X SSC, 10% dextran sulfate at pH 7) and the telomeric oligonucleotide in the same hybridization buffer at 50 ng/μl (after unsuccessful attempts between 15 and 30 ng/μl).

The chromosome preparations were denatured at 72°C +- 2°C for 1 min 15 sec in 70% (v/v) formamide, 2 X SSC (pH 7), dehydrated through 70% (-20°C), 80% and 100% ethanol series at room temperature (2 min each), and air-dried. Hybridizations of microsatellite and telomeric sequences were conducted with 40 μl per slide under large coverslips (24 x 40 mm). Slides were incubated overnight in a moist chamber at 37°C. For post-hybridization washes, slides were incubated for 2 min in 0.4 X SSC, 0.3% Tween 20 at 62°C, 1 min in 2 X SSC, 0.1% Tween 20 at room temperature. The chromosomes were counterstained in DAPI (4',6-diamidino-2-phenylindole)-antifade solution (Vectashield, Vector Laboratories, Peterborough, UK).

Microscope analysis

Microscopic images were obtained using a Zeiss AXIO Imager.M1 with a CoolSNAP ES camera. Photomicrographs were processed with the Genus software for karyotyping and FISH-Imaging (Leica Microsystems).

RESULTS

Phylogenetic tree

A 1080 bp long fragment of the mitochondrial cytochrome b was aligned. A total of 347 variable sites were identified for all samples, out of which 157 were parsimony informative (i.e. shared by at least two different sequences). Using the BIC (Tamura *et al.*, 2011), the optimal model of sequence evolution was GTR+G (General Time Reversible; Tavaré, 1986). This model distinguishes between transition and transversion. The G parameter indicates that non-uniformity of evolutionary rates among sites is modelled by using a discrete Gamma distribution. This model has been used for the subsequent analysis. The phylogenetic relationships observed were congruent with previously published results (Lavoué *et al.*, 2000). The phylogenetic tree obtained (Fig. 2) was rooted with *Gymnarchus niloticus* (Gymnarchidae) a family closely related to the mormyrids. *Ivindomyrus opdenboschi* is the sister group of this clade, and *Brienomyrus* sp. is the sister group to these three, although both these branches are less supported (67% and 97% respectively). *Stomatorhinus cf. ater* is the sister group to this larger clade, with high robustness (98%).

Karyotypes and FISH mapping

When describing chromosomes and their formulae, we use the following nomenclature and abbreviations:

m: metacentric chromosomes
sm: submetacentric chromosomes
a: acrocentric chromosomes (including subtelocentric and telocentric chromosomes)
p arms: short arms of acrocentric chromosomes
FN: fundamental number (arm number)
ITS: interstitial telomeric signal
NORs: nucleolar organizer regions corresponding to the 45S rDNA (including the 18S rDNA).

In order to obtain an overview of all the Giemsa stained and C-banded karyotypes of the species under study, and thus facilitate their comparison, the species have been plotted onto the branches of the phylogenetic tree obtained as explained in the "materials and methods" paragraph (Fig. 3).

Unfortunately, most specimens analysed were adults (*S. walkeri*) or juveniles of very small size and so produced a limited number of chromosome preparations, thus making it impossible to repeat experiments. This explains the irregularities in the chromosome quality, especially after being submitted to denaturation in C-banding and FISH experiments. Not enough chromosome preparations were available to perform double FISH experiments to explore, for example, the relative position of telomeric signals versus ribosomal or microsatellites ones.

Among specimens analysed within each species, no chromosomal variability and no morphologically differentiated sex chromosomes could be recorded.

Among hybridized microsatellites, none produced any hybridization signal, except the sequence $(GAA)_{10}$.

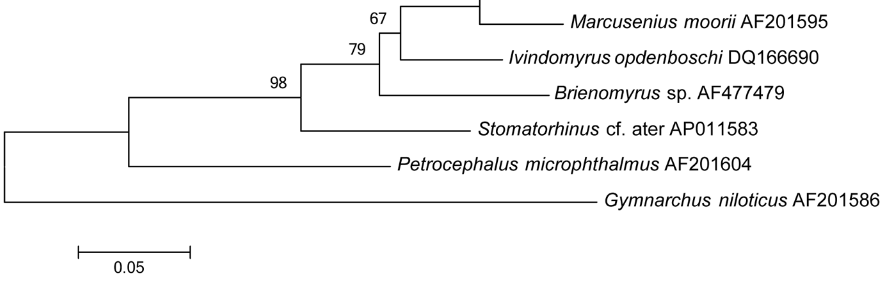
99
67
79
98
Gnathonemus petersii AF201585
Marcusenius moorii AF201595
Ivindomyrus opdenboschi DQ166690
Brienomyrus sp. AF477479
Stomatorhinus cf. ater AP011583
Petrocephalus microphthalmus AF201604
Gymnarchus niloticus AF201586
0.05

Figure 2. - Consensus tree built from the mitochondrial cytochrome b partial sequences, based on the maximum likelihood method. Numbers above the branches are percentages of bootstrap values based on 2000 replicates. *Gymnarchus niloticus,* a Gymnarchid fish close to the mormyrids was used to root the tree.

Gnathonemus petersii

The karyotype is composed of 2n = 48 chromosomes (2sm + 18 m + 28a), FN = 68. C-bands are small and essentially located in centromeric regions but at least four chromosome pairs also exhibit intercalated bands in their long arms, in particular the large (sm) pair, and several acrocentric pairs (Fig. 3). In several metaphase plates, the 18S rDNA genes could be localized in pericentromeric position in a large sm chromosome and not in its homologous (Fig. 4A, B). The telomeric probe hybridized on almost all chromosomes, but generally not at their four telomeres. No interstitial telomeric signal (ITS) could be recorded (Fig. 4C). The microsatellite $(GAA)_{10}$ is abundant and mainly located in telomeric positions with an intercalary band in pericentromeric position in the large sm pair (Fig. 4D).

Marcusenius moorii

The karyotype is composed of 2n = 50 (4sm + 46a), FN = 54. C-bands are less clearly marked than in *G. petersii* and essentially found in the centromeric regions of all chromosomes, but observed in interstitial position in the long arms of the large sm pair in several metaphase plates (Fig. 3). The 18S rDNA genes are located in the narrow DAPI-negative pericentromeric region of a medium size acrocentric pair (Fig. 4E, F). The telomeric probe produced a weak signal on all chromosomes but not at their four telomeres and no ITS could be recorded (Fig. 4G). The microsatellite $(GAA)_{10}$ is abundant, mainly located in telomeric positions with few intercalary bands in pericentromeric position in some chromosome pairs (Fig. 4H).

Ivindomyrus opdenboschi

The karyotype is composed of 2n = 50 (2sm + 10m + 38a), FN = 62. A clear C-banding for this species could not be obtained: C-bands are found in the centromeric regions of all chromosomes and one band is located intercalary, in the long arms of the large sm pair (Fig. 3). The 18S rDNA genes are located in the DAPI-negative short arms of a medium sized bi-armed element, but due to over-denaturation, it is difficult to assign it to a precise chromosome pair (Fig. 4I, J). The telomeric probe produced strong telomeric signals in all chromosomes but not all telomeres. In some

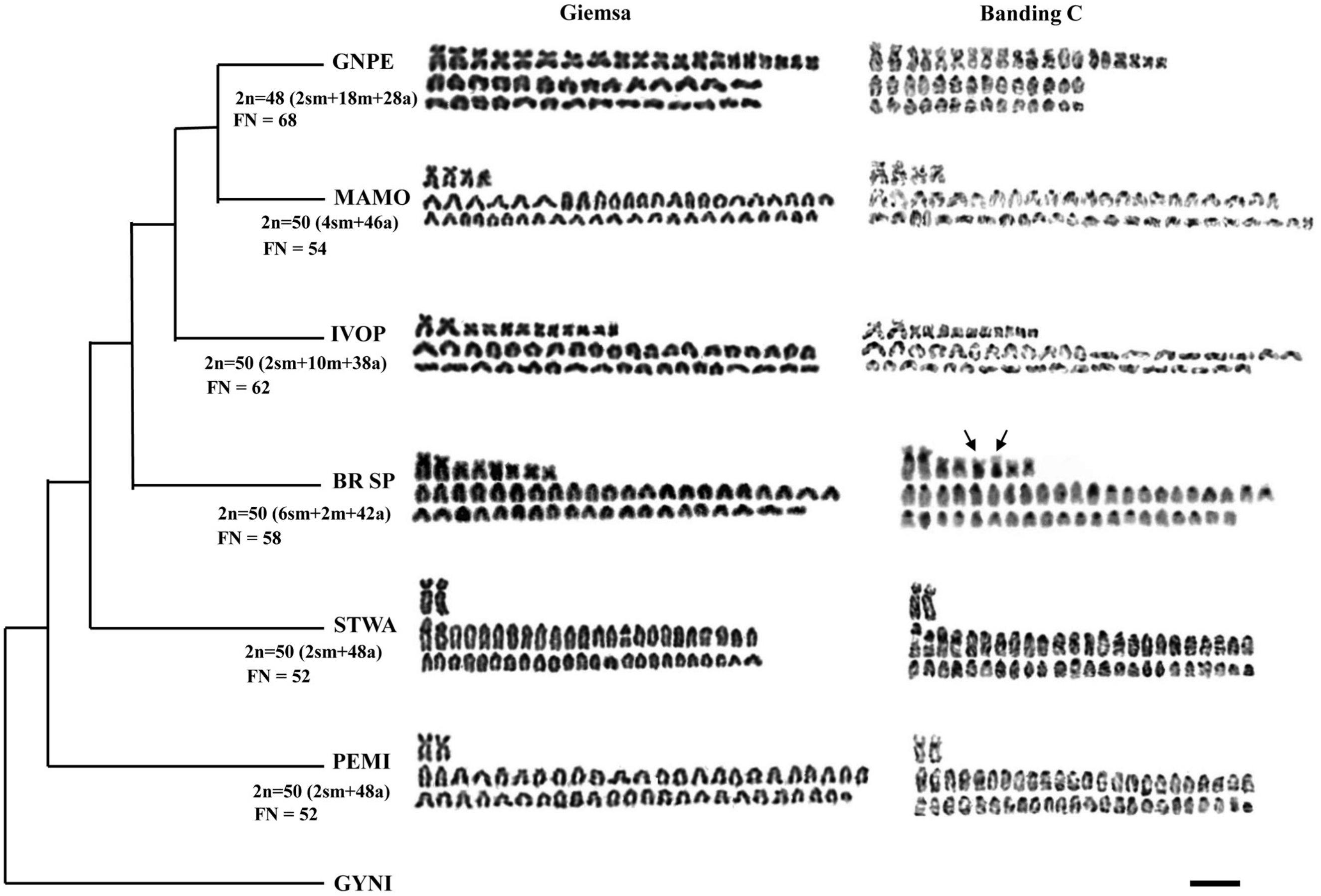

Figure 3. - Karyotypes of *G. petersii*, GNPE, *M. moorii*, MAMO, *I. opdenboschi*, IVOP, *Brienomyrus* sp., BR SP, *S. walkeri*, STWA, and *P. microphthalmus*, PEMI, after conventional Giemsa staining and C-banding. Scale bar = 10 µm.

acrocentric pairs, they can be located in long arms, pericentromeric position (Fig. 4K). The microsatellite $(GAA)_{10}$ is abundant and mainly located in telomeric positions with few intercalary bands in pericentromeric position (Fig. 4L).

***Brienomyrus* sp.**

The karyotype is composed of 2n = 50 (6sm + 2 m + 42a), FN = 58. C-bands are found in the centromeric regions of all chromosomes and no ITS was detected. The long arm of the third submetacentric is entirely C-band positive (Fig. 3, arrows). The 18S rDNA genes are located in terminal position on the long arms of submetacentric chromosomes (certainly corresponding to the third sm pair in size). These arms are DAPI-negative in their distal part (Fig. 5A, B). The telomeric probe produced weak signals in some telomeres, but not in all chromosomes (Fig. 5C). The microsatellite $(GAA)_{10}$ is abundant and mainly located in telomeric positions with few intercalary bands in pericentromeric position (Fig. 5D).

Stomatorhinus walkeri

The karyotype is composed of 2n = 50 (2sm + 48a), FN = 52. C-bands are found in the centromeric regions of all chromosomes and many in the interstitial, pericentromeric or telomeric regions of acrocentric pairs. In particular, the first pair of acrocentrics is strongly heteromorphic due to a pericentromeric constriction of unequal size within this pair (Fig. 3). The 18S rDNA genes are heteromorphic in size, DAPI-negative, and located in the long arms of an acrocentric chromosome pair (Fig. 5E, F). This chromosome pair probably corresponds to the first acrocentric pair in the karyotype, as shown in figure 3. With the FISH protocol used for the other mormyrid species, the telomeric probe produced almost no signals in the telomeres (Fig. 5G). We tested two different batches of telomeric probe at several concentrations (up to 50 ng/µl) and less stringent post-hybridization washes (not detailed in the method) without obtaining any further hybridization spots. The microsatellite $(GAA)_{10}$ is,

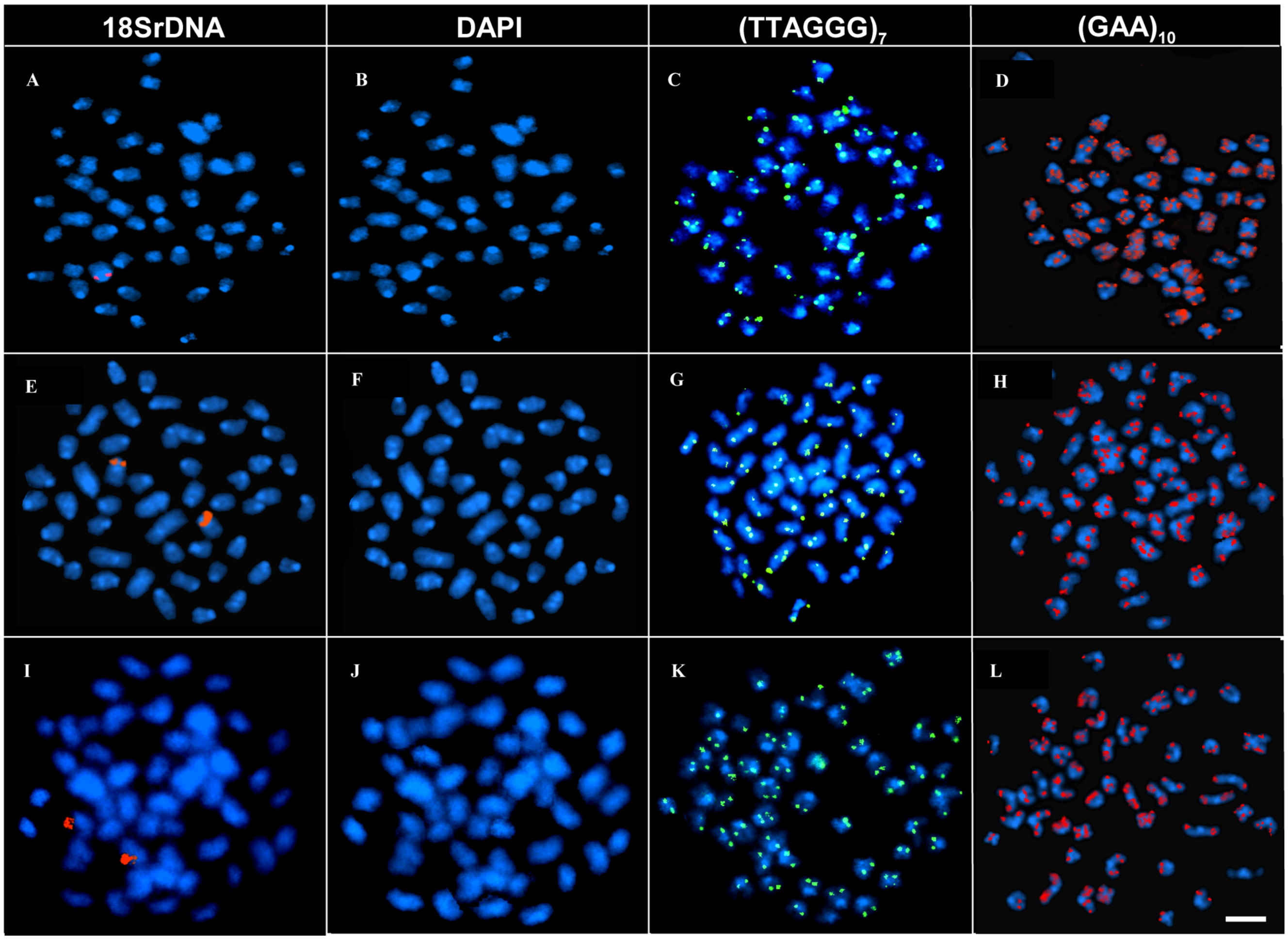

Figure 4. - Metaphase plates of *G. petersii* (A, B, C, D), *M. moorii* (E, F, G, H) and *I. opdenboschi* (I, J, K, L), showing FISH patterns of the 18S rDNA probe, indirectly DIG-labelled, detected with rhodamine; DAPI counterstaining on the same metaphase plate; patterns of the Atto 488 labeled $(TTAGGG)_7$ probe; patterns of the Cy3 labeled $(GAA)_{10}$ satellite probe. Scale bars = 10 µm.

on the contrary, the most abundant among species examined (Fig. 5H).

Petrocephalus microphthalmus

The karyotype is composed of 2n = 50 (2sm + 48a), NF = 52. C-bands are found in the centromeric regions of all chromosomes and many in the interstitial, pericentromeric or telomeric regions of acrocentric pairs (Fig. 3). The 18S rDNA genes correspond to DAPI-negative regions. They are located in the short arms of two acrocentric pairs (Fig. 5I, J). The telomeric probe hybridized on all chromosomes in telomeric positions and no ITS could be recorded. Two acrocentric chromosomes bear giant telomeres in their short arms (Fig. 5K). The microsatellite $(GAA)_{10}$ is abundant and located in centromeric and telomeric positions (Fig. 5L).

DISCUSSION

Karyotypes and C-banding patterns

Based on the molecular syntenies of several distantly related vertebrate proto-karyotypes recently sequenced, the ancestral teleost karyotype has been proposed to be either 2n = 48 (Jaillon *et al.*, 2004; Kohn *et al*, 2006), or 2n = 50 (Nakatani *et al.*, 2007). Many teleost fish groups have conserved chromosome numbers, with 48-50 chromosomes composed of majority of acrocentric pairs and few meta-submetacentric ones. This is the case for *Petrocephalus microphthalmus* and *Stomatorhinus walkeri*. Both species have the same chromosome diploid number (2n = 50) and identical chromosome formulae (2sm + 48a). Their single sm pair, which is slightly larger than all other chromosomes in both karyotypes, arose either from a pericentric inversion within a large acrocentric chromosome, leaving the diploid chromosome number (2n = 50) unchanged, or from a centric fusion between two small acrocentric elements, which

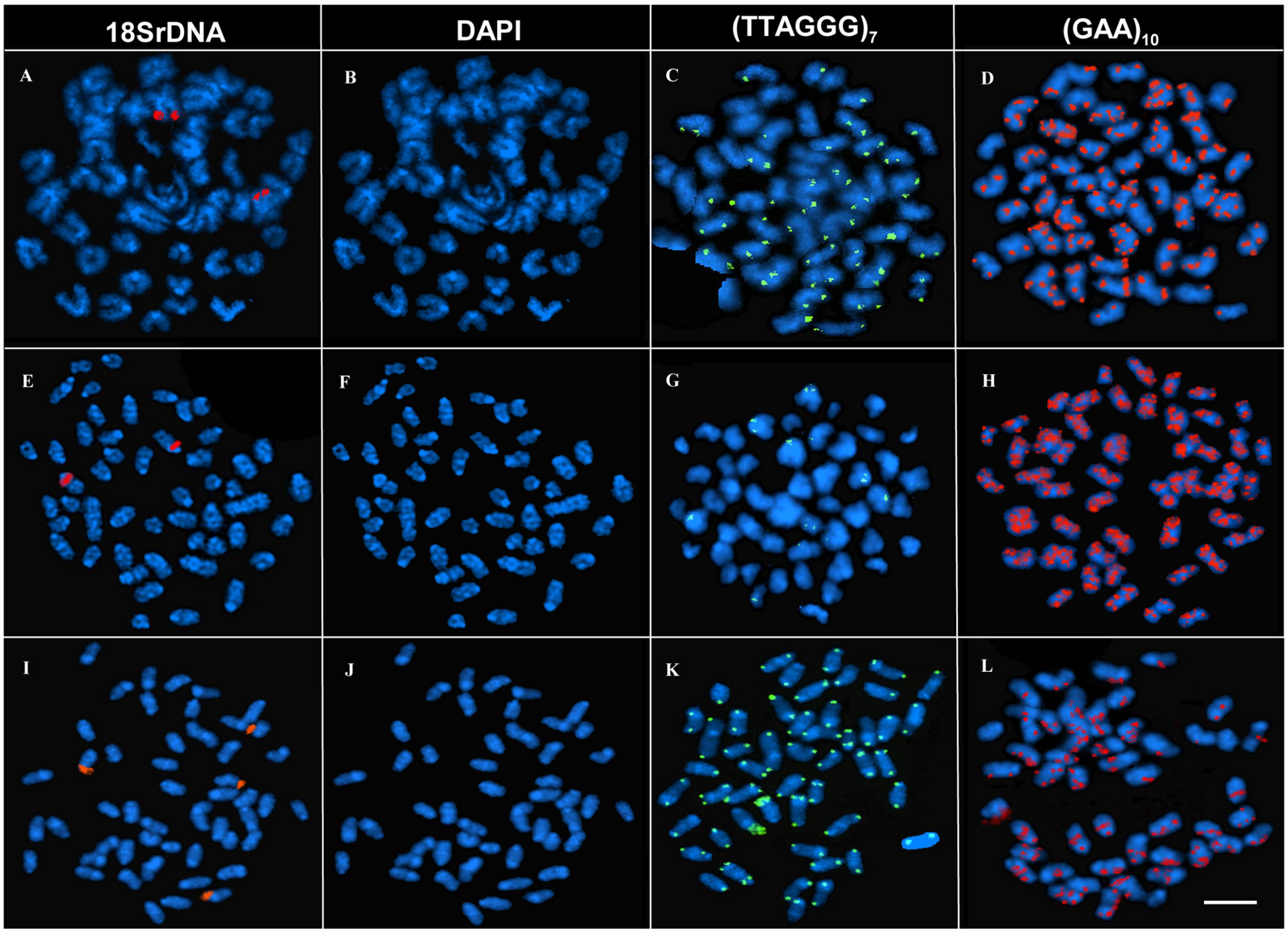

Figure 5. - Metaphase plates of *Brienomyrus* sp. (A, B, C, D), *S. walkeri* (E, F, G, H) and *P. microphthalmus* (I, J, K, L), showing FISH patterns of the 18S rDNA probe, indirectly DIG-labelled, detected with rhodamine; DAPI counterstaining on the same metaphase plate; patterns of the Atto 488 labelled $(TTAGGG)_7$ probe; patterns of the Cy3 labelled $(GAA)_{10}$ satellite probe. Scale bars = 10 μm.

would also entail a fission event, in order to maintain the unchanged diploid number (2n = 50). These sm pairs show slightly different centromere positions in the two species, suggesting they are not syntenic. Both species exhibit a rather complicated C-banding pattern, with numerous centromeric, intercalary and telomeric bands, indicating a high rate or intra-chromosomal rearrangements, even if numbers and formula remained unchanged. The four other species forming a clade, *Brienomyrus* sp. (2n = 50), *Ivindomyrus obtenboschi* (2n = 50), *Marcusenius moorii* (2n = 50) and *Gnathonemus petersii* (2n = 48) show practically unchanged chromosome numbers, but specific chromosome formulae involving an increasing number of sm-m elements or gradually decreasing size, and correlatively increased FNs. This typically evokes an active mechanism of karyotype diversification involving successive pericentric inversions within acrocentric elements. Beside these chromosomal rearrangements, the C-bands are essentially restricted to centromeres, or entirely cover one chromosome arm in one pair (arrows) in *Brienomyrus* sp.*,* while in the three other species they are distributed in all centromeres, but can also be intercalary of telomeric in some chromosome pairs, and could be interpreted as fingerprints or more complex intra-chromosomal rearrangements.

Few specimens could be analysed per species, and we could not detect any intra-specific change in the karyotypes, nor morphologically differentiated sex chromosomes. Since we had at disposal only one species per genus, we cannot estimate the rate of chromosomal diversification within each of these genera. The karyotype of another species of *Brienomyrus* (*B. (Marcusenius) brachistius)* was published by Uyeno (1973), who found, on the basis of a single specimen, a diploid number of 48 (1m + 4sm + 43a) with a heteromorphic chromosome pair. *Brienomyrus* sp. has a diploid number of 50 chromosomes, and a different chromosome formula (2m + 6sm + 42a) but the proportion of m-sm elements in the karyotype remains very close between the two species. Uyeno (1973) also published the karyotype of a species identified as *Gnathonemus petersii* with a diploid number of 48 chromosomes (10m + 6sm + 32 a) while we found (2sm + 18 m + 28a). However, it is impossible to establish whether this difference is due to intra-specific chromosomal polymorphism, to hybridization with another species or is populational, because the animal analyzed by Uyeno was purchased from a tropical fish store without neither a clear geographic origin, nor preserved voucher. Our *G. petersii* specimens also come from a shop and have no clear geographic origin, but they did not show any intraspecific chromosomal polymorphism and with foresight we both kept specimen vouchers and used DNA barcoding for their precise characterization (Tab. I).

18S ribosomal gene patterns

The 18S ribosomal DNA probe has been FISH-mapped onto the chromosomes of the six mormyrid species under study to localize the nucleolus organizer regions (NORs), where the major (45S = 18S + 5.8S + 28S) ribosomal RNA (rRNA) genes (rDNA) are located. NOR numbers and distribution have been established by strictly molecular approaches in a relatively small number of teleosts (1.3%) as reviewed in Gornung (2013). They can be single (in most cases) or multiple, and in most cases, “centromeric-terminal” in acrocentric chromosomes. In the six mormyrid species examined, they show a striking diversity of FISH patterns: in *Petrocephalus microphthalmus*, they are terminal in the p arms of two acrocentric chromosome pairs; in *Stomatorhinus walkeri*, they are heteromorphic, and pericentromeric in the largest acrocentric pair; in *Brienomyrus* sp., they are terminal in the long arms of a sm pair (the third in size); in *Ivindomyrus obdenboschi* they appear terminal in a small bi-armed chromosome pair; in *Marcusenius moorii*, they are pericentromeric, and in a small acrocentric pair; and in *Gnathonemus petersii*, they are pericentromeric in a large sm chromosome and not visible in its homologue, possibly due to an unequal number of copies within this chromosome pair. NOR size polymorphism within the same chromosome pair is frequently observed in teleost fish. This NOR position in *G. petersii* could be possibly a synteny with the position observed in *Brienomyrus* sp. However, this is not supported by the topology of the mitochondrial cyt b phylogenetic tree, which suggests, if we rely this topology, that chromosomal rearrangements leading to these apparently identical patterns occurred separately, and all other 18S rDNA patterns found in the other mormyrid species as well.

Since we have examined only one species per genus, we have no indication whether the observed patterns are specific, or generic, but since they are clearly not syntenic from one species to another, they also reveal an intensive genome re-patterning among the mormyrid species under study.

Telomeric $(TTAGGG)_7$ and microsatellite $(GAA)_{10}$ patterns

The telomeric probe $(TTAGGG)_7$ revealed strong hybridization signals in all species except *S. walkeri* where almost no signal could be detected. Since all FISH experiments with this probe were performed in the same conditions, it suggests that the *S. walkeri* genome has a lower copy number of this telomeric sequence than the other species and / or contains degenerate telomeric repeats. In the other species, the observed signals are not often visible on the two telomeric extremities of the chromosomes and appear more frequently on q arms than in p arms, where they are probably too short to be detected. In fish, the chromosomal distribution of the telomeric DNA sequences is described in approximately 74 actinopterygian species (Okalewicz, 2013). The length of their telomeric DNA arrays shows interspecific variation and ranges from 2 to 25 kb (Lund *et al*., 2009; Downs *et al*., 2012). In *G. petersii*, *M. moorii* and *I. opdenboschi*, ITS are frequently observed in pericentromeric position of m and sm pairs and can be interpreted as fingerprints of pericentric inversions. In *P. microphthalmus*, two giant telomeric hybridization spots are observed on the p-arms of an acrocentric chromosome pair. Although we could not verify this hypothesis by double FISH with a ribosomal probe, it is reasonable to assume that these giant telomeres are co-located with the major rDNA sequences, in one of the two NOR-bearing pairs, as observed in several other teleost species (Gornung *et al*., 2004; Rossi *et al*., 2005). One possible hypothesis (Guillen *et al*., 2004) is that the ITS when interspersed with the major rDNA sequences, epigenetically inactivate NORs.

Among the seven oligonucleotides containing microsatellite sequences, only the $(GAA)_{10}$ microsatellite produced a clear and abundant hybridization pattern. It was essentially distributed in heterochromatic (pericentromeric and telomeric) chromosomal regions of all the Mormyrid species under study and sometimes intercalates in chromosome arms. Thus, we can speculate that this microsatellite is co-located with telomeric repeat regions and have thus co-evolved. This finding is in agreement with models predicting accumulation of repetitive DNA sequences in low recombination regions (Charlesworth *et al*., 1994; Stephan and Cho, 1994).

In conclusion, among the six mormyrid genera investigated, chromosome formulae have obviously rapidly diverged, mainly involving pericentric inversions. NORs exhibit an amazing diversity of positions and C-band distributions also reveal an important re-patterning, which could be used for inter-generic or interspecific comparison. A more refined analyse, by means of zoo-fish with chromosome-specific paints, would be necessary to ascertain interspecific chromosomal homologies and thus contribute to a better understanding of the mechanisms of chromosomal evolution within the Mormyrid family.

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